

to addition of IL-10 alone. Consistent with a Tr1 phenotype, approximately 50% of the IL-10 positive cells were also positive for IFN- γ . Further efforts are underway to determine the percentage of IL-10+IL-4- and IL-10+IL-2- cells at the single cell level. In order to definitively establish the percentage of Tr1 cells differentiated in vitro, we plan to perform limiting dilutions (after the secondary stimulation) in the presence of allogeneic feeder-cell mixture and to analyze the profile of cytokine production at the clonal level.--

IN THE CLAIMS:

Please cancel Claims 8, 9, and 15-18 without prejudice. Please amend Claims 1 and 10, as indicated.

1. (Once Amended) A method to induce differentiation of a naïve CD4 $^{+}$ T cell to a Tr1 cell comprising contacting the T cell with an appropriate amount of interferon- α (IFN- α).

10. (Once Amended) The method of Claim 1, wherein the contacting is in combination with an appropriate amount of IL-10.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned, "Version with markings to show changes made."

REMARKS

Claims 1-18 are pending. Claims 1-9 and 11-18 are rejected. Claim 10 is objected to. Claims 8, 9, and 15-18 are canceled without prejudice. The Specification is amended as requested by the Examiner. Claim 1 is amended to encompass a specific subset of T cell. Support for this amendment can be found, e.g., on page 10, lines 19-28, of the Specification. Claim 10 is amended to correct claim dependency. Applicants believe that no new matter is added by the foregoing

amendment.

I. Objection to the Specification.

The Examiner objected to a missing page number for a cited reference on page 14, line 8. The Examiner further objected to the Specification for not indicating the US patent number of an application cited in the Specification. Applicants have amended the Specification accordingly and request this objection be withdrawn.

II. Objection to Claim 10.

The Examiner objected that Claim 10 as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form. Applicants believe that Claim 1, from which Claim 10 depends, is currently allowable in view of Applicants' amendment as noted above. Applicants submit that Claim 1 is now in allowable form and as such the objection to Claim 10 is moot.

In view of the foregoing, withdrawal of this objection is respectfully requested.

III. Rejection of Claims 1-9 and 11-14 under 35 U.S.C. §112, First Paragraph.

The Examiner rejected Claims 1-9 and 11-14 under 35 U.S.C. §112, first paragraph, on the basis that of enablement. Claims 8 and 9 are canceled and the rejection is therefore moot as to these claims.

The Examiner alleges that the claims are drawn to methods of using IFN- α to induce T cell precursor differentiation, while Lin, *et al.*, teaches that IFN- α blocks T cell precursor differentiation (page 3, lines 6-7, of Office action).

Applicants respectfully disagree. Applicants submit that Lin, *et al.*, is narrowly drawn to "an early progenitor state" that is "very early in B and T cell development," where the inhibitory effects of IFN- α have a "window of sensitivity to IFN- α 2/ α 1 . . ." (page 83, column 1; page 84, columns 1 and 2 of Lin, *et al.*). As amended, Claim 1, encompasses differentiation of a naive CD4+ T cell into a Tr1 cell using IFN- α . Applicants conclude that the narrow situation described by Lin, *et al.*, does not necessarily apply to Applicants' invention.

The Examiner further alleges that IFN- α and IL-10 are both necessary for

differentiation, while Lin, *et al.*, does not teach that both these factors are needed. (page 4, lines 12-14, of Office Action). Applicants respectfully disagree. Applicants submit that addition only IFN- α alone is needed when using, e.g., cord blood CD4+ T cells (page 9, lines 15-18, of Specification).

The Examiner further finds that neither the Specification nor the prior art provides guidance to indicate that adding an "antigen" or "alloantigen," or that "proliferation using IL-15" would have the claimed effect (page 3, lines 14-18, of Office action)..

Applicants respectfully disagree. Applicants submit that the effects of antigen or alloantigen are both described in the Specification, where guidance is provided on the use of antigens via a mixed lymphocyte reaction (MLR) to stimulate proliferation of naive CD4+ T cells. See, e.g., page 8 of Specification. It is further shown that this proliferation is inhibited by Tr1 cells. See, e.g., page 8, lines 13-20; and page 30, lines 12-27, of the Specification. Furthermore, use of alloantigens to stimulate proliferation of Tr1 cells is described on page 8, lines 21-27, of the Specification. Applicants also submit that the use of IL-15 to proliferate Tr1 cells is described (page 15, lines 3-4, and page 28, lines 9-18, of the Specification). Use of anti-IL-10 and anti-TGF- β antibodies as proliferative agents is also detailed (page 8, lines 21-22). Applicants conclude that the Specification enables the skilled artisan to make and use the present invention.

In view of the foregoing, the rejection of Claims 1-9 and 11-14 under 35 U.S.C. §112, first paragraph, is overcome and withdrawal of this rejection is respectfully requested.

IV. Rejection of Claim 16 under 35 U.S.C. §112, Second Paragraph.

The Examiner rejected Claim 16 under 35 U.S.C. §112, second paragraph, for indefiniteness. Claim 16 is canceled and the rejection is therefore moot as to this claim. Withdrawal of this rejection is respectfully requested.

V. Rejection of Claims 15-18 under 35 U.S.C. §102(b).

The Examiner rejected Claims 15-18 under 35 U.S.C. §102(b), as being anticipated by Avice, et al. (1998) J. Immunol. 161:3408-3415. As noted above, Claims 15-18 are canceled and the rejection is therefore moot as to these claims. In view of the foregoing, Applicants submit that the basis for this rejection has been overcome. Withdrawal is respectfully requested.

Conclusion

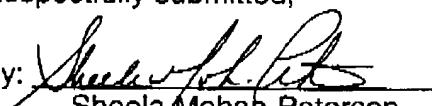
Applicants' current response is believed to be a complete reply to all the outstanding issues of the Office action. Further, the present response is a bona fide effort to place the application in condition for allowance or in better form for appeal. Accordingly, Applicants respectfully request reconsideration and passage of the claims to allowance at the earliest possible convenience. Should the Examiner deem allowance inappropriate at this time, Applicants respectfully request an interview be granted with the undersigned to consider any issues.

Applicants believe that no additional fees are due with this communication. Should this not be the case, the Commissioner is hereby authorized to debit any charges or refund any overpayments to DNAX Deposit Account No. 04-1239.

If the Examiner believes that a telephonic conference would aid the prosecution of this case in any way, please call the undersigned.

Respectfully submitted,

Dated: December 12, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADEIn the specification:

The paragraph beginning on page 3, line 14, has been amended as follows:

T-regulatory cells have an important role in peripheral tolerance, but it has been difficult to isolate cells with suppressive activity in vitro and to define their mechanism of action. A CD4⁺ T-regulatory cell subset has been described which is able to suppress antigen-specific immune responses in vitro and in vivo. See, e.g., USSN 07/846,208, filed March 4, 1992; USSN 08/643,810, filed May 6, 1996, now U.S. Pat. No. 6,277,635; and Groux, et al. (1997) Nature 389:737-742; each of which is incorporated herein by reference. Type 1 T-regulatory (Tr1) cells are defined, in part, by their unique cytokine profile: they produce high levels of IL-10, significant levels of TGF-β and IFN-γ, but no IL-4 or IL-2. Herein, it is investigated whether in vitro differentiation of human Tr1 cells from naive CD4⁺ T cells is regulated by cytokines. It is shown that in cord blood T cells, IFN-α induces differentiation of a population of cells with a Tr1-like profile of cytokine production. In contrast, with peripheral blood T cells, both exogenous IL-10 and IFN-α were required for differentiation of Tr1 cells. Cultures with Tr1 cells had a reduced proliferative capacity in response to polyclonal activation, and a suppressed response to alloantigens. Suppression of the alloantigen response was mediated in part by IL-10 and TGF-β. The present invention is based, in part, on the definition of conditions for in vitro differentiation of human Tr1 cells. This will facilitate further characterization of this unique T-cell subset and enable their clinical use as cellular therapy to induce tolerance to foreign proteins, e.g., alloantigens.

The paragraph beginning on page 14, line 5, has been amended as follows:

A number of experiments were designed to determine the effects of IL-10, IFN-α, and IL-15 on the differentiation of IL-10-producing T cells. Efforts have focussed on aspects of the differentiation system described by Sornasse, et al.

(1996) *J. Exp. Med.* 184:473-[xxx] 483) which involves co-culture of CD4+ T cells with irradiated L-cells, expressing CD32, CD58, and CD80, in the presence of anti-CD3, IL-2, and/or IL-15, and polarizing cytokines. Following two rounds of stimulation, cells are collected, stimulated with α CD3 and α CD28, and analyzed by intra-cytoplasmic staining and ELISA for the production of IL-10, IL-4, IL-2, and IFN- γ . Experiments were initiated with CD4+ T cells derived from cord blood, which cells have an innate ability to produce high levels of IL-10. Addition of IFN- α resulted in a significant, e.g., 5-6 fold, increase in the percentage of IL-10-positive cells compared to addition of IL-10 alone. Consistent with a Tr1 phenotype, approximately 50% of the IL-10 positive cells were also positive for IFN- γ . Further efforts are underway to determine the percentage of IL-10+IL-4- and IL-10+IL-2- cells at the single cell level. In order to definitively establish the percentage of Tr1 cells differentiated in vitro, we plan to perform limiting dilutions (after the secondary stimulation) in the presence of allogeneic feeder-cell mixture and to analyze the profile of cytokine production at the clonal level.

In the claims:

1. (Once Amended) A method to induce differentiation of a naïve CD4⁺ T cell to a Tr1 cell comprising contacting the T cell [precursor] with an appropriate amount of interferon- α (IFN- α) [, wherein said contacting induces differentiation to a Tr1 cell].
10. (Once Amended) The method of Claim [8] 1, wherein [said] the contacting is in combination with an appropriate amount of IL-10.